510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k051714

B. Purpose for Submission:

New device

C. Measurand:

Cholyglycine, Bile Acids

D. Type of Test:

Diazyme's Total Bile Acids assay measures serum bile acids concentration enzymatically. The assay is a spectrophotometric method, which can be adapted to most automated clinical chemistry analyzers.

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Total Bile Acids Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR §862.1177, Cholyglycine Test System

2. Classification:

   Class II

3. Product code:

   KWW

4. Panel:

75 (Chemistry)

H. Intended Use:

1. Intended use(s):

   See Indications for use.

2. Indication(s) for use:

   Diazyme Total Bile Acids Assay is intended for the in vitro quantitative determination of total bile acids (TBA) in human serum samples.

   Total Bile Acids Assay contains a bile acid calibrator. The calibrator is designed to be used with the assay for the quantitative determination of TBA in serum.

   Total Bile Acids Assay control is designed to be used with the assay for the quantitative determination of TBA in serum.

3. Special conditions for use statement(s):

   For Prescription use only.

4. Special instrument requirements:

   All tests were done using the Hitachi 717 Auto-analyzer instrument and Diazyme Total Bile Acid assay kit.

I. Device Description:

The Diazyme's Total Bile Acids assay is a clinical test kit, intended for the quantitative determination of total bile acids in serum by an enzymatic method. The Diazyme's Total Bile Acids assay is comprised of Reagent 1, Reagent 2, and a calibrator.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Trinity Biotech Bile Acids Reagent

2. Predicate 510(k) number(s):

   k872296

3. Comparison with predicate:

The table below indicates the similarities and differences between the Diazyme's Total Bile Acid Assay and its predicate device Trinity Biotech Trinity Bile Acids Assay (k872296)

Similarities		
Characteristic	New Device Diazyme's Total Bile Acids Assay (k051714)	Predicate Device Trinity Biotech Trinity Bile Acids (k872296)
Indications for Use	For the quantitative in vitro determination of bile acid in human serum.	For the quantitative in vitro determination of bile acid in human serum
Format	Liquid	Lyophilized
Principle	Enzymatic cycling 405 nm	Enzymatic cycling 530 nm
Type of Test	Quantitative	Quantitative
Specimen Type	Serum	Serum
Product Type	Reagent, Calibrator, Control	Reagent, Calibrator, Control

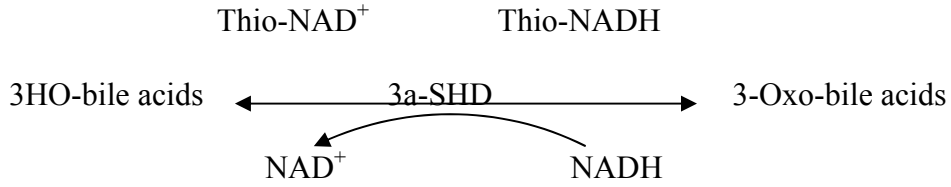
K. Standard/Guidance Document Referenced (if applicable):

None Referenced.

L. Test Principle:

In the presence of Tion-NAD, the enzyme 3-α-hydroxysteroid dehydrogenase (3-α-HSD) converts bile acids to 3-keto steroids and Thio-NADH. The reaction is reversible and 3-α-HSD can convert 3-keto steroids and Thio-NADH to bile acids and Thio-NAD. In the presence of excess NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring specific change of absorbance at 405nm.

The assay mechanism is shown below:



M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The intra-assay precision coefficient of variation was evaluated with two different bile acid levels (low concentration = 8 μM and high concentration = 23 μM).

Cycling TBA Intra precision Assay (on Hitachi 717)

Run	Diazyme Control (μM)	Diazyme Control (μM)
1	8.098	23.691
2	7.645	23.213
3	7.968	23.828
4	7.531	23.386
5	7.619	23.625
6	8.202	22.785
7	8.242	23.610
8	7.968	23.997
9	8.264	23.474
10	7.319	23.367
11	8.141	23.781
12	7.752	23.651
13	8.099	23.404
14	8.331	23.957
15	8.122	23.158
16	7.987	23.775
17	8.263	23.368
18	7.647	23.167
19	8.025	23.641
20	7.345	23.118
Mean (μM)	**7.93**	**23.50**
STD Dev	**0.31**	**0.30**
CV (%)	**3.86**	**1.29**

Determined by running 20 replicates of two samples of various levels of total bile acid in one run.

The inter-assay precision coefficient of variation was evaluated by testing the two level specimens (low concentration = 8 μM and high concentration = 23 μM) in 20 runs. All tests were done using the Hitachi 717 Auto-analyzer instrument and Diazyme Total Bile Acid assay kit.

Cycling TBA inter assay precision (on Hitachi 717)

Determined by two runs per day each of three level samples of total bile acid in ten different days.

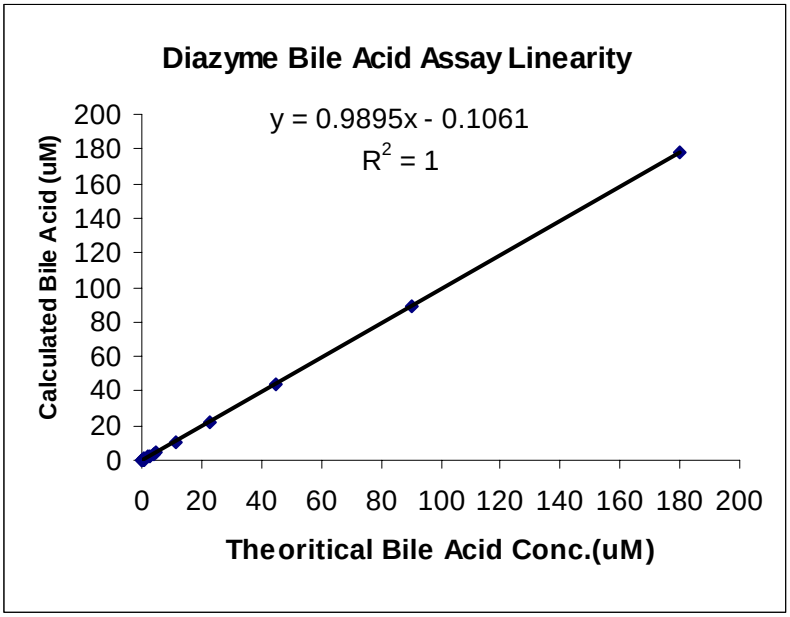
Runs	sample #1	AVG	STD	%CV	sample #2	AVG	STD	%CV
1	22.4	22.91	0.61	2.65	7.9	8.12	0.24	2.99
	22.6				8.1			
2	22.8				8			
	22.8				7.97			
3	22.4				8.1			
	23.6				7.9			
4	23.9				8.13			
	22.7				8.2			
5	23.4				8.4			
	23.2				8.1			
6	23.4				7.9			
	23.6				8.1			
7	22.2				8.2			
	23.2				8.4			
8	23.4				8.7			
	22.8				8.3			
9	23				8.4			
	23.3				8.1			
10	22				7.9			
	21.5				7.6			

b. Linearity/assay reportable range:

According to the sponsor, data to support the claimed lower range linearity between 0 and 5 µM were prepared by dilution of a buffer sample spiked with 180 µM Bile Acid. The sample was diluted using buffer in proportions to create a dilution series. The dilution series resulted in 12 different levels of linearity test samples with expected and theoretical bile acid values depicted below. Calculated bile acid values were derived from an average of 4 replicates.

Theoretical Bile Acid, (uM)	Calculated Bile Acid, (uM)
0	0.05
0.25	0.27
0.5	0.48
1	0.84
2	1.84
3	2.86
4	3.79
5	4.92

11.3	10.8
22.5	22.2
45	44.2
90	89.1
180	178



c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Reagents Stability:

To establish the shelf life and to predict the expiration date of the kit, accelerated stability tests were conducted. A representative number of lots (3) of the Diazyme Total Bile Acid Assay reagents were used for this study. The data obtained is shown in the table below and indicated that the reagents are stable up to an equivalent of at least one year (12 months) when stored at 4°C. The acceptance criterion is an absorbance change of 15% or less. All three lots of reagents had satisfied the criteria for passing.

TBA cycling stability for liquid reagents at 37°C

Sample (μM)	*Lot No.*	*Day 0 ΔA405nm On UV-Spec*	*Day 5 ΔA405nm On UV-Spec*	*Change (%)*	*Day 7 ΔA405nm On UV-Spec*	*Change (%)*

50	BA07404	0.0783	0.0760	- 2.9	0.0697	- 10.1
50	BA07504	0.0742	0.0731	- 1.5	0.0689	- 7.1
50	BA07905	0.0750	0.0728	- 2.9	0.0692	- 7.7

Calibrator Stability

The results depicted below demonstrate that the calibrator is stable for at least 7 days when stored at 37°C. The acceptance criterion is an absorbance change of 5% or less. The lot used in this study had satisfied the criteria for passing.

TBA cycling stability for liquid calibrator at 37°C

Lot No.	*Day 0 ΔA405nm On UV-Spec*	*Day 5 ΔA405nm On UV-Spec*	*Change (%)*	*Day 7 ΔA405nm On UV-Spec*	*Change (%)*
BA05104	**0.0689**	**0.0680**	**- 1.0**	**0.0675**	- 1.7

The data above indicated that the calibrator is stable up to an equivalent of at least one year (12 months) when stored at 4°C.

Control Stability

To establish the shelf life and to predict the expiration date of the control set, real time and accelerated stability tests were carried out in a study.

Lyophilized Control Accelerated Stability Study

The data obtained is shown in the table below and indicated that the lyophilized controls are stable up to an equivalent of at least 9 months when stored at 4°C.
The acceptance criterion is an accuracy CV% of 10% of less. Both levels of control in this accelerated test met the criteria for passing.

Control	Bile Acid value (μM) at 37°C on Day 0	Bile Acid value (μM) at 37°C on Day 5	Bile Acid value (μM) at 37°C on Day 7
Level 1	30.3	30.6	29.4
Level 2	110.4	113.4	111.3

Reconstituted Control Real Time Stability Study
The data obtained is shown in the table below and indicated that the reconstituted control is stable up to 7 days when stored at 4°C. The acceptance criterion is an accuracy CV% of 10% of less. The control in this test met the criteria for passing.

Control	Bile Acid value (µM) at 4°C on Day 0	Bile Acid value (µM) at 4°C on Day 5	Bile Acid value (µM) at 4°C on Day 7
Level 1	30.3	31.0	29.8

d. Detection limit:

To demonstrate the limit of quantification of the total bile acid measurement by the Diazyme TBA Enzymatic Assay, test sample with bile acids of 10 µM was diluted with Solution A to obtain the sample concentrations of 6, 4, 3, 2, 1, and 0.5 µM. The limit of quantification is defined as the lowest concentration having a CV < 15%. The samples were analyzed by the Diazyme TBA Enzymatic Assay by eight replicates.

Sample type: human serum dilution with 50mM MES buffer
Target concentrations/range: 1-6
Number of measurements per concentration: 8
Solution A: 50 mM MES buffer, pH 6.0
Sensitivity method: lowest analyte concentration where %CV is acceptable
Acceptance criteria: lowest analyte concentration where CV < 15%

Average data from all eight samples are shown below:

Theoretical BA µmol/L	Observed BA µmol/L	SD µmol/L	% CV
6	5.97	0.14	2.27
4	4.01	0.22	5.55
3	2.98	0.16	5.33
2	1.97	0.15	7.47
1	0.99	0.13	13.37
0.5	0.53	0.08	15.8

The results demonstrate that the limit of quantification for the Diazyme TBA Enzymatic Assay is 1 µmol/L.

e. Analytical specificity:

To study the effect of potential interferences in the Diazyme TBA Enzymatic assay, agents like bilirubin, hemoglobin, triglyceride, and ascorbic acid were spiked into normal human serum to be used as samples. In this study, 3 replicates of each agent were evaluated.

% interference is calculated using the following equation:

$$\frac{\text{Spiked sample} - \text{non-spiked sample}}{\text{Non-spiked sample}} \times 100 = \% \text{ interference}$$

Acceptance criteria: calculated % interference must be less than 5%

Interference Agents:

1. To study the effect of bilirubin on the bile acid measurement, a serum sample with bile acid level of 22 µM, was spiked with bilirubin to achieve bilirubin serum level of 50 mg/dL. This concentration is in addition to the bilirubin concentration found in the normal serum sample.

2. To study the effect of hemoglobin on the bile acid measurement, a serum sample with bile acid level of 22 µM, was spiked with hemoglobin to achieve a hemoglobin serum level of 500 mg/dL. This concentration is in addition to the hemoglobin concentration found in the normal serum sample.

3. To study the effect of ascorbic acid on the bile acid measurement, a serum sample with bile acid level of 22 µM, were spiked with ascorbic acid to achieve serum level of 50 mg/dL. This concentration is in addition to the ascorbic acid concentration found in the normal serum sample.

4. To study the effect of lipid on the bile acid measurement, a serum sample with bile acid level of 22 µM, was spiked with lipid to achieve lipid serum level of 750 mg/dL. This concentration is in addition to the lipid concentration found in the normal serum sample.

Interference Data:

Bilirubin mg/dL	**Total Bile Acid µmol/L**	**Interference %**
0	22.25 $\pm$ 0.21	-
50	22.40 $\pm$ 0.23	0.6

Hemoglobin mg/dL	Total Bile Acid µmol/L	Interference %
0	22.25 ± 0.21	-
500	22.25 ± 0.19	0.0

Ascorbic Acid mg/dL	Total Bile Acid µmol/L	Interference %
0	22.25 ± 0.21	-
50	21.35 ± 0.14	0.96

Triglyceride mg/dL	Total Bile Acid µmol/L	Interference %
0	22.25 ± 0.21	-
750	22.20 ± 0.10	0.0

f. Assay cut-off:

Not applicable for this type of device.

2. Comparison studies:

a. Method comparison with predicate device:

The accuracy study comparing the Diazyme Total Bile Assay with the Predicate Trinity' s Bile Acid method presented a slope of 1.15, correlation coefficient 0.99, and y intercept of 0.89. I felt the 15% bias compared to the predicate device was an issue because the Diazyme method did not show good correlation with the predicate device in the normal expected range of 0-10 µM.

There were 8 samples (sample # 4, 5, 6, 8, 14, 16, 19, and 23 in the correlation data list) whose TBA values were significantly different from the values determined by the predicate method. The sponsor indicated that they were aware of this discrepancy and additional studies were performed. The sponsor came to the conclusion that the discrepancies noted were not caused by the inaccuracy of the Diazyme TBA method but was because of the poor anti-interference capacity and low sensitivity of the NBT/formazan based predicate method.

The sponsor presented the following 5 points to demonstrate that the new enzymatic cycling based total bile acids assay gives more accurate results for lipemic and hemolytic samples than does the predicate method.

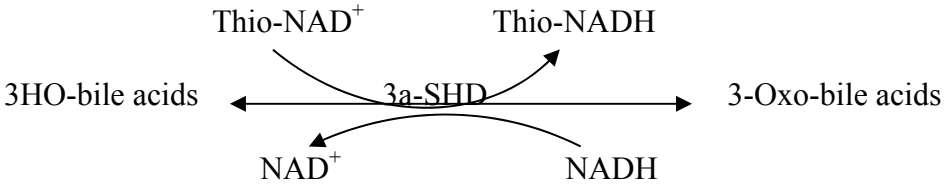
1. Difference in assay mechanisms: enzyme cycling versus non-cycling and rate assay versus end point assay.

It is well known that there are two major drawbacks of the enzymatic, colorimetric bile acids assay method (the predicate method) using nitro blue tetrazolium as color indicator coupled with NAD. One is low sensitivity and the other is significant interferences from hemolytic and lipemic serum samples. The assay mechanism of the predicate method is depicted as below:

3HO-bile acids + NAD^+ —3a-SHD→ 3-Oxo-bile acids + NADH + H^+

NADH + H^+ + tetrazolium salt —diaphorase→ NAD + formazan (540 nm)

To overcome these drawbacks of the NBT/formazan based enzymatic bile acids assay, a more sensitive and high capacity of anti-interference method has been developed. This new method (the Diazyme method) utilizes an enzyme cycling based mechanism that allows each bile acid molecule in the patient serum to produce multiple molecules of Thio-NADH for detection. Through repeatedly oxidation and reduction reaction cycles, the indicator Thio-NADH is significantly amplified. This rate based assay significantly reduced lipemic and hemolytic interferences in comparison with the end point based predicate assay. The assay mechanism is shown below:



2. Drawbacks of the predicate method described in the literatures

The two major drawbacks, low sensitivity and significant interferences by lipemic and hemolytic samples are well documented in the literatures. For example, in Ueda et al's 1994 patent (U.S. 5,286,627), it is described that the enzymatic colorimetric total bile acids assay (the predicate method) is liable to be influenced by bilirubin, and is low sensitivity (see the attachment #1).
In another literature published in 1991 in the *Journal of bioluminescence and chemiluminescence*, Lekhakula et al described a discrepancy of total bile acids levels determined by two different methods. Lekhakula found that the BNT method (predicate method) gave 4 times higher TBA values for those hypertriglyceridemic and hypercholesterolemic sera (see the attachment #2). This observation was similar to what Diazyme has observed.

3. Direct interference comparison between the enzyme cycling method and the predicate method

Diagnostic Chemicals Limited (DCL), a Canada based major clinical diagnostic reagent company who currently distribute Diazyme's total bile acids reagent in U.S.

has made a head to head comparison of interferences by hemoglobin and triglycerides on TBA assays. As shown in the attachment #3, the predicate method is significantly affected by hemoglobin and triglycerides. The former causes under estimation of TBA value whereas the later causes over estimation of TBA values. In contrast, the enzyme cycling method is not significantly affected by both hemoglobin and triglycerides

4. The cause of the discrepancy was confirmed experimentally

We measured the triglycerides and hemoglobin contents of the 8 problematic serum samples, and found that all the samples showed higher TBA values in the predicate method were lipemic samples and all the samples showed lower TBA values in the predicate method were the hemolytic samples as shown in the table below. These observations are consistent with the literature descriptions and DCL's experimental findings.

		TBA (umol/L)		Hemoglobin	Triglycerides
Sample #	Sample ID	Diazyme	Predicate	mg/dL	mg/dL
4	10372162	2.52	8.94	16	222.2
5	10372163	2.59	0.75	165	96.5
6	10372164	4.59	9.08	8	156.2
8	10372166	3.95	9.64	10	171.4
14	10372172	2.64	7.78	24	247.1
16	10372178	8.13	4.42	136	124.8
19	10372181	1.28	6.29	15	284.0
23	N62064	4.64	0.51	160	105.6

b. Matrix comparison:

Not applicable**.**

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable**.**

*b. Clinical specificity**:***

Not applicable**.**

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off**:**

   Not applicable**.**

5. Expected values/Reference range:

   Serum 0 – 10 µM

   The expected range presented in the proposed package insert was established from scientific literature with references listed below:

1. Toshihide, Shima, et al. (2000) Interferon Treatment For Chronic Hepatitis C. Journal of Gastroenterology and Hepatology, 15, 294-299.
2. Youichi, Komiyama, et al. (1982) Microassay of Serum Bile Acids by an Enzymatic Cycling Method. Chem. Pharm. Bull., 30 (10), 3798-3799.
3. B. Franz and J. Ch. Bode (1974) Total Plasma Bile Acid Concentration in Chronic Hepatitis and Cirrhosis; Fasting Values and Effect of Intraduodenal Bile Salt Administration. Kin. Wschr., (52) 522-526.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.